

Article

Antioxidant Seasonal Changes in Soilless Greenhouse Sweet Peppers

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Abstract: This study was commissioned to study the effect of the growing season on the antioxidant components of greenhouse sweet pepper crops, which is of scientific interest because of their possible beneficial health effects. The total antioxidant activity (estimated by ferric reducing antioxidant power-FRAP assay) major antioxidants (ascorbic acid, phenolics and carotenoids) and taste fruit quality characteristics (soluble solids, titratable acidity, dry matter and sugars) were recorded in soilless-grown sweet pepper cultivars of red, orange, yellow and green color at four harvesting season months, i.e., February (winter), May (spring), July (summer) and October (autumn). The results showed seasonal variations in antioxidant components and activity of pepper fruits. In most cases measured parameters showed higher values in spring (May) and summer (July) compared with winter (February) and autumn (October) growing seasons. This study indicates that during late autumn and winter, lower levels of solar irradiance, ultraviolet radiation and temperature in Mediterranean greenhouses can be insufficient to stimulate phytochemicals production in peppers; thus, plant–light interception must be more actively managed.

Keywords: *Capsicum annuum* L.; colored sweet peppers; antioxidant activity; phenolics; ascorbic acid; carotenoids; solar and ultraviolet radiation; soilless culture

1. Introduction

Peppers are among the most consumed vegetables worldwide. In the United States consumption of fresh bell peppers (*Capsicum annuum* L.) increased up to 20% the last decade and averaged 11.2 pounds per person in 2018 [1]. Bell peppers have become one of the most important cultivated fruiting vegetable in Mediterranean greenhouses. Particularly, solanaceous crops (tomato, pepper and eggplant) constitute about 60% of greenhouse-cultivated areas, which are often cultivated in soilless culture to enhance yield, product quality, water use efficiency and sustainability [2,3]. To date, peat-based substrates are most widely used in fruiting vegetable production systems, although rockwool is still the dominant soilless culture system in Europe [4]. Furthermore, alternative ecofriendly substrates (e.g., biowaste materials) with a lower carbon footprint coupled with soilless culture systems may be considered as a useful tool in sustainable greenhouse horticulture [5].

Recently, Mediterranean sweet peppers gained a growing interest to produce brightly colored (e.g., red, yellow, orange) fruits throughout the year based on the favorable climatic conditions (high radiation and mild autumn and winter temperatures) of the region [6], modern soilless culture technologies and the good marketability of the product. In this context, EU [7] has established specific marketing standards for sweet peppers. However, many consumers have additional quality requirements, which can go beyond legislation and standards [8]. Thus, the interest in foods of plant

origin as a source of phytochemicals, increases throughout the years [9]. Specifically, the consumption of natural antioxidant compounds coming from fruits and vegetables such as phenolics, ascorbic acid and carotenoids have been associated with prevention of chronic diseases (e.g., cardiovascular disease and different forms of cancer) due to their ability to neutralize free radicals in the human body [10,11]. Peppers are among vegetable crops that are considered as naturally abundant in plant phytochemicals and their composition is of major importance for the beneficial health effects of the product [12,13]. As already mentioned peppers are widely grown in Mediterranean greenhouses, however, the lack of information regarding seasonal influences on the accumulation of dietary antioxidants in colored pepper fruits represents a drawback (e.g., in diets based on antioxidant intake). It is relevant that in other crops such as tomatoes and lettuce, seasonality can affect their antioxidant composition [14,15].

It is well known that genetic and environmental factors may directly affect the antioxidant composition of plant parts [16,17]. Particularly, under Mediterranean climatic conditions, increased solar irradiation and mild temperatures during winter months can affect plant antioxidant content and eventually fruit quality [18,19]. Although different species may have different responses [20], there is a general notion that solar ultraviolet radiation (UV total radiation 280–400 nm) has relevant biological effects on agroecosystems and induce the accumulation of phenolic compounds in the plant [14,19,20]. However, global UV fraction (i.e., ratio of the UV to global solar radiation) is highly dependent on variations in the concentration of clouds, water vapor and aerosols in the atmosphere and may vary from 2.0% to 9.5% [21,22]. Such environmental factors impact the quality of greenhouse vegetables. For example, a positive correlation between light levels and levels of secondary metabolites such as ascorbic acid in sweet peppers has been reported [8]. In accordance, the increase levels of Ultraviolet B (UVB) radiation (280–315 nm) enhanced several defense compounds such as carotenoids and flavonoids in bell peppers [23]. Ultraviolet A (UVA) radiation (315–400 nm) also enhanced the amounts of secondary metabolites, soluble carbohydrates, free amino acids and proteins in greenhouse peppers [24]. Consequently, it is assumed that changes in global solar and UV radiation and air temperature levels at different harvesting seasons, may affect the antioxidant components of greenhouse soilless-grown peppers, which is of scientific interest because of their possible beneficial effects on human health.

To document this response, this study was designed to evaluate seasonal effects in total antioxidant activity (estimated by ferric reducing antioxidant power-FRAP assay) and important antioxidant compounds (i.e., ascorbic acid, phenolics, carotenoids) so as in other fruit quality characteristics (soluble solids, titratable acidity, pH, dry matter, reducing sugars) in soilless sweet peppers of red, orange, yellow, and green color, in Mediterranean greenhouses.

2. Materials and Methods

2.1. Plant Material and Agronomic Features

Data was collected from the same plants of a year round sweet pepper crop, giving harvests in 2017, at greenhouse facilities of Agricultural Research Institute of Cyprus (34°94' N, 33°19' E, altitude 40 m). Three colored pepper (*Capsicum annuum* L.) cultivars (Agroglobal, Hungary), namely red (*cv. Castello*), orange, (*cv. Donat*), yellow (*cv. Solero*) and one green local variety (*cv. Glikes*) were grown on rockwool substrates (Grodan Company, Denmark; slabs dimensions 100 cm × 20 cm × 7.5 cm). Substrates were placed into Polygal-gutters (Mapal Plastics, Israel) 12-m long, which were supported by metal frames in 12 single rows. Each experimental unit consisted of one Polygal-gutter planted with 24 plants. Three replications (one Polygal-gutter per replication) for each cultivar were randomly arranged in three blocks. The plants were vertically supported ('V' system) giving a planting density of 2.0 plants m⁻² [25]. Colored fruits (red, orange, yellow) ripened on the vine following the mature green stage. The crop was transplanted on rockwool slabs 3 months prior harvesting, which started in February and terminated in October 2017. Harvested fruits of each experimental unit were weighted and counted to determine fresh yield and average weight of the fruit. Total marketable fruit yield was

the combined total of Extra Class and Class I according to EU marketing standards [7]. For quality analysis, fruits were sampled at four harvesting times, particularly in February (winter), May (spring), July (summer) and October (autumn).

The irrigation schedule was controlled by Fertimix hydroponic head unit (Galgon, Kfar Blum, Israel) and adjusted to light conditions [26]. The start of irrigation was depended from light sums according to the growth stage (1500–2800 kJ/m²) targeting a leaching fraction of about 20%. Drip emitters delivered the nutrient solution directly to the root zone of pepper plants. Electrical conductivity (EC) and pH values were monitored in both irrigation and drainage water. The target EC levels of the irrigation nutrient solution were adjusted in response to radiation differences (± 0.3 dS/m; higher EC at low radiation and lower EC at high radiation). The hydroponic fertigation head prepared a nutrient solution (NS) for growing soilless peppers in Mediterranean greenhouses with NS composition originating from the literature [2]. The following NS was delivered to the plants at the vegetative stage: 5.4 mM K⁺, 4.65 mM Ca²⁺, 1.6 mM Mg²⁺, 1.2 mM NH₄⁺, 13.7 mM NO₃[−], 1.2 mM H₂PO₄[−], 1.85 mM SO₄^{2−}, 15 μ M Fe as Fe-EDDHA, 10 μ M Mn, 5 μ M Zn, 0.8 μ M Cu, 30 μ M B, and 0.5 μ M Mo. Corresponding EC and pH values were 2.20 dS/m and 5.6, respectively. At the reproductive stage the plants were fed with the following nutrient solution: 5.8 mM K⁺, 4.5 mM Ca²⁺, 1.40 mM Mg²⁺, 0.6 mM NH₄⁺, 13.0 mM NO₃[−], 1.2 mM H₂PO₄[−], 1.75 mM SO₄^{2−}, 15 μ M Fe as Fe-EDDHA, 10 μ M Mn, 5 μ M Zn, 0.8 μ M Cu, 30 μ M B, and 0.5 μ M Mo. The EC and pH values of this NS were 2.10 dS/m and 5.6, respectively.

2.2. Greenhouse Facilities and Climatic Data

The experiment was conducted in a North–South oriented greenhouse with a total ground area of 216 m², with cutter height 3.50 m, ridge height 5.26 m, spans width 6 m and total length 18 m. The gable end and side walls were covered with double-walled polycarbonate and the roof was covered with a common polyethylene film (88% light global transmission, 55% light diffused transmission and 88% thermal efficiency). In each greenhouse span there was a single continuous rooftop window for natural ventilation. In addition, evaporative cooling was performed by a fan-pad system consisted of four fans, two at each span and a wetted pad. The greenhouse floor was completely covered by a white, water permeable polypropylene sheet.

External climatic parameters measured were air relative humidity (RH_o, %) and temperature (T_o, °C) (Sensor type PT 100; Galcon, Kfar Blum, Israel) and net solar radiation (G_h, kJ/m²) with a pyranometer at 3 m above the greenhouse (Sensor type TIR-4P; Bio Instruments Company, Chisinau, Moldova). The same types of sensors were used for monitoring relative humidity and air temperature within the greenhouse. All measurements were recorded every 30 s on a data logger system (Galileo controller; Galcon, Kfar Blum, Israel) and a ten-minute average was estimated. Vapor-pressure deficit (VPD) was estimated based on greenhouse air temperature and relative humidity. The mean daily value of ultraviolet radiation over a month was calculated based on global solar radiation, following Equation (1). According to this formula [27], the hourly and daily values of both radian fluxes are highly correlated with a general linear relationship of the following form providing coefficients of determination of R² always greater than 0.91 for hourly and 0.88 for daily fittings in the case of Cyprus.

$$G_{uv} = aG_h \quad (1)$$

where G_{uv} is the solar global ultraviolet radiation (kJ/m²); G_h is the solar global radiation (kJ/m²); a is the slope corresponding to measurements.

2.3. Fruit Quality Measurements

The fruit quality characteristics (i.e., ascorbic acid, sugars, total soluble solids, pH, titratable acidity and dry matter) were determined at commercial maturity stage (Figure 1) in randomly selected samples excluding outliers, from each experimental unit. The edible part of the fruit was

homogenized and soluble solids ($^{\circ}$ Brix; Atago PR-1, Tokyo, Japan), pH (Mettler Toledo, Switzerland), titratable acidity (titration with sodium hydroxide solution to pH 8.2, % citric acid), and fruit dry matter (g/100 g FW, drying at 70 $^{\circ}$ C) were recorded. The ration between total soluble solids and titratable acidity was calculated (TSS/TA). The content of fruits in ascorbic acid (mg AA/100 g FW) and reducing sugars (mg Glucose+Fructose/g FW) were determined by a Merck RQflex reflectometer. Briefly, ascorbic acid reduces yellow molybdophosphoric acid to phosphormolybdenum blue that is determined reflectometrically as reducing sugars after enzymatic conversion with glucose-6-phosphate dehydrogenase and diaphorase according to the company protocols (Merck, Darmstadt, Germany). For the determination of the total phenolic content and antioxidant activity, subsamples of fruits were kept-frozen at -30° C until the date of analysis. Quantitative determination of phenolic substances was performed in fruits samples (10 g) homogenized with 25 mL acidified acetone (acetone: water: acetic acid 70:29.5:0.5, v:v:v), following the Folin–Ciocalteu procedure [28]. The absorbance of the reaction mixtures (0.25 mL extract, 2.5 mL FolinCiocalteu’s reagent (previously diluted 1:10 with deionized water) and 2 mL 7.5% Na_2CO_3) after 5 min at 50 $^{\circ}$ C was measured at 760 nm (UV-Vis spectrophotometer Helios Zita, Thermo Fisher Scientific, USA). The results were expressed in gallic acid equivalents (mg GAE) per g of fresh weight, using a calibration curve (GAE/g FW) [29]. For the determination of the antioxidant capacity of pepper fruits by the ferric reducing antioxidant power method (FRAP; [30,31], sample extracts (100 μL) were mixed with 3 mL FRAP reagent (1:1:10 mixture of 20 mM FeCl_3 , 10 mM TPTZ and 0.3 M acetate buffer at pH 3.6) and after 4 min at 37 $^{\circ}$ C the absorbance at 593 nm was recorded. Ascorbic acid (AA) was used as standard and the results were expressed per g of fresh weight ($\mu\text{mol AA/g FW}$) as previously described [29]. Chlorophyll content was determined in green fruit samples blended with 80% acetone measuring the absorbance of the supernatant at 648 and 664 nm [29]. Total carotenoids content in colored fruits extracts (hexane: acetone: ethanol 50:25:25, v:v:v) was determined at 450 nm following concentration calculations as previously reported [32]. The results were expressed as mg β -carotene per g of FW.

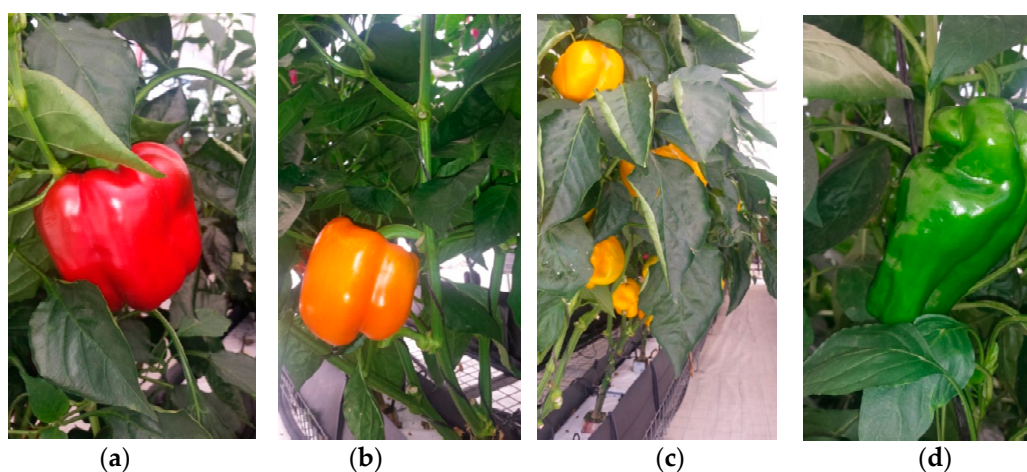


Figure 1. Fruit maturity at the time of harvest in (a) red; (b) orange; (c) yellow; (d) green sweet pepper (*Capsicum annuum* L.) cultivars grown in greenhouse soilless culture.

2.4. Statistical Analysis

Experimental layout in the greenhouse consisted of three replicates for each cultivar arranged in a randomized complete block design. SAS software system (ver. 9.2, Cary, NC, USA) was used for analysis of variance (ANOVA) for all traits studied and means were separated using DMRT at 5% level of significance. Pearson correlation coefficients between antioxidant variables studied were calculated.

3. Results

3.1. Greenhouse Microclimate and External Climatic Data

The monthly mean values, of outdoor climate data (i.e., air temperature and relative humidity) and inside greenhouse microclimate; global solar radiation and calculated ultraviolet radiation are presented in Table 1. The monthly variability of both radiant fluxes, Gh and Guv, is shown in Figure 2.

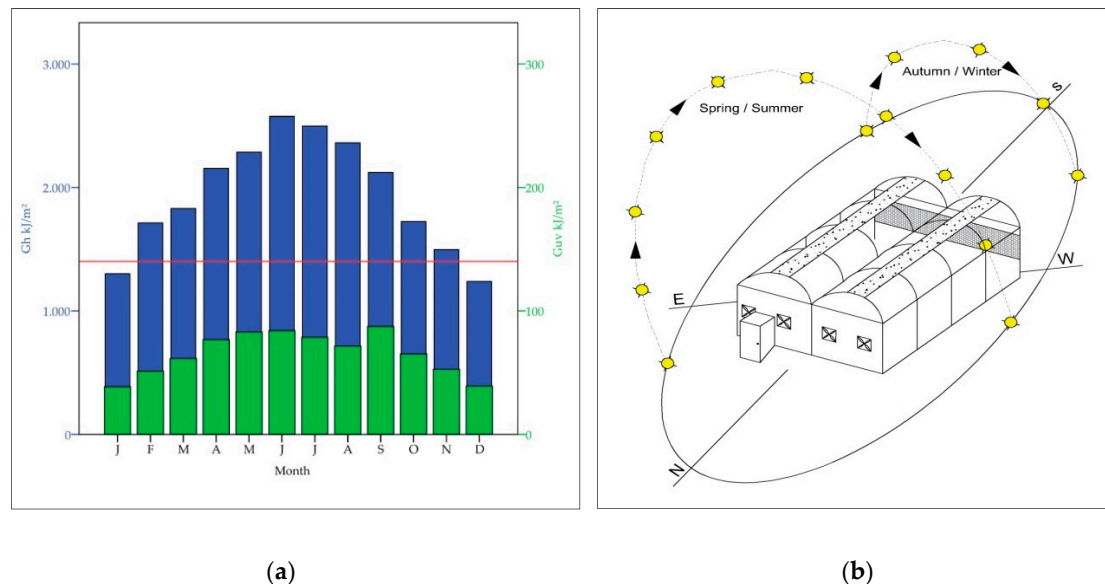


Figure 2. (a) Monthly means of global solar and ultraviolet radiation (kJ/m^2 ; the bars are in relation but not proportional to the data they encode); (b) Sun orientation during the experiment; Straight-line embedded in the graph (a) represents minimum radiation requirements for cultivation of thermophilic vegetable species in Mediterranean greenhouses [33].

The mean estimated values kJ/m^2 (\pm standard deviation) of Gh and Guv were respectively 1378 (799.99) and 42 (24.08) in winter (D-J-F), 2087 (1216.19) and 74 (43.48) in spring (M-A-M), 2451 (1222.93) and 77 (38.76) in summer (J-J-A) and 1788 (976.99) and 69 (39.42) in autumn (S-O-N). Seasonal variations of Guv value followed seasonal variations of Gh. Particularly, higher Guv values observed during summer and lower values at winter; as affected by yearly length of a day and the solar zenith angle. However, from Table 1 we can observe that, despite the decrease of Gh from August to September by 15%, Guv values increased by 17%. The line in Figure 2a represents minimum radiation requirements for cultivation of thermophilic vegetable species in Mediterranean greenhouses during N-D-J according to the literature [33].

Table 1. Monthly mean values (\pm standard deviation) of outdoor climate data and inside greenhouse microclimate for daylight hours.

	J	F	M	A	M	J	J	A	S	O	N	D
Gh	1075 (810)	1509 (1022)	1801 (1096)	2269 (1234)	2528 (1211)	2838 (1135)	2746 (1144)	2515 (1148)	2151 (1095)	1681 (987)	1251 (857)	972 (751)
Guv	32 (24)	45 (30)	60 (37)	81 (44)	91 (44)	92 (37)	86 (36)	76 (34)	89 (45)	63 (37)	44 (30)	31 (24)
To	14.8 (4.2)	20.5 (1.7)	22.6 (4.2)	26.0 (2.2)	28.6 (2.5)	31.7 (2.1)	33.5 (3.1)	30.0 (2.9)	28.5 (3.0)	22.7 (3.4)	20.5 (2.6)	12 (3.2)
RHo	55.7 (14.7)	49.3 (12.5)	46.8 (8.4)	48.4 (5.9)	53.5 (9.5)	65.8 (8.5)	63.9 (14.8)	72.0 (11.5)	55.4 (11.0)	47.2 (15.0)	49.2 (11.9)	53.0 (9.8)
Ti	21.0 (4.9)	23.7 (2.4)	24.5 (2.9)	25.4 (3.5)	26.2 (2.9)	27.9 (2.0)	28.0 (2.0)	27.9 (2.0)	24.2 (2.2)	23.8 (4.7)	23.0 (3.6)	21.8 (2.3)
RHi	69.8 (11.8)	59.2 (12.5)	60.2 (11.3)	61.5 (7.8)	67.2 (6.6)	69.8 (5.3)	65.3 (9.1)	75.1 (14.3)	60.3 (12.8)	62.9 (14.7)	54.9 (11.7)	61.5 (6.3)
VPD	0.9 (0.5)	1.2 (0.4)	1.1 (0.2)	1.1 (0.3)	1.1 (0.3)	1.3 (0.4)	1.4 (0.2)	1.1 (0.3)	1.2 (0.3)	1.2 (0.2)	1.3 (0.2)	0.9 (0.3)

Gh, global solar radiation (kJ/m^2); Guv, ultraviolet radiation (kJ/m^2); To, outside greenhouse air temperature ($^{\circ}\text{C}$); RHo, outside greenhouse air relative humidity (%); Ti, inside greenhouse air temperature ($^{\circ}\text{C}$); RHi, inside greenhouse air relative humidity (%); VPD, inside greenhouse air vapor pressure deficit (kPa).

3.2. Antioxidants and Other Fruit Quality and Yield Parameters

Season and cultivar were in most cases significant sources of variation (Table 2). Because of some interactions observed between season and cultivar, data were graphically presented within each cultivar (Figure 3). The antioxidant activity (FRAP values; $\mu\text{mol AA/g FW}$) of the pepper cultivars tested showed higher values in spring (May) and summer (July) compared with winter (February) and autumn (October) (Figure 3). An increase was also observed for total phenolics (GAE/g FW) during May compared with February in orange and yellow cultivars, however, in red and green cultivars the increase was not significant ($p < 0.05$; Figure 3). Accordingly, ascorbic acid content (mg AA/100 g FW) showed higher value in May and July and lower in February and October in all cases (Figure 3). Similarly, sugars (mg Glucose + Fructose/g FW) were accumulated at higher levels during May and July compared with the other two months in red, orange and yellow pepper fruits, whereas in green fruits the values observed were not differentiated with harvest time (Figure 3). Changes in total soluble solids ($^{\circ}\text{Brix}$) with harvesting time followed alterations of the sugar content as may be expected. However, in some cases (orange cultivar) differences were not consistent (Figure 3). The titratable acidity (% citric acid) was higher during July compared with February for red, orange and yellow cultivars, whereas no variation was observed among harvest times for the green cultivar. Yet importantly, carotenoids content (mg β -carotene/g FW) at harvesting times May and July was enhanced in colored pepper fruits in relation with the other two months depending on the cultivar (Figure 3). On the contrary, total chlorophyll (a + b) content in the green cultivar remained unaffected by the growing season (Figure 3), so as the dry matter content in most of the cases. Similarly, the estimated ratio total soluble solids to titratable acidity (TSS/TA) was not differentiated among harvest months in the cultivars tested. Overall, total marketable fruit yield (kg/m^2) and mean fruit weight (g/fruit) was greater in colored peppers in relation to the green cultivar (Figure 4). On the contrary, more fruits per m^2 were produced by the green than the rest of the colored cultivars (Figure 4). Last but not least, FRAP values were highly correlated ($p < 0.001$) with phenolics ($r = 0.81$) and ascorbic acid ($r = 0.84$), whereas pigment phytochemicals had a lower influence to the reducing potential. In addition, phenolics were highly correlated with ascorbic acid ($r = 0.77$) so as both with reducing sugars ($r = 0.60$ and $r = 0.83$, respectively).

Table 2. Analysis of variance table and levels of significance ($p < 0.05$, $p < 0.01$, $p < 0.001$).

Source	F Probability								
	FRAP	Ph	AA	Sug	TSS	TA	Car	Chl	DM
Season	0.0010	0.6085	<0.0001	<0.0001	0.0012	<0.0001	<0.0001	0.1736	0.1724
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S \times C	0.0305	0.0042	0.0534	0.1100	0.6260	0.9321	0.0035	<0.0001	0.0016

Antioxidant activity (FRAP), phenolics (Ph), ascorbic acid (AA), sugars (Sug), total soluble solids (TSS), titratable acidity (TA), carotenoids (Car), total chlorophyll (Chl) and dry matter (DM).

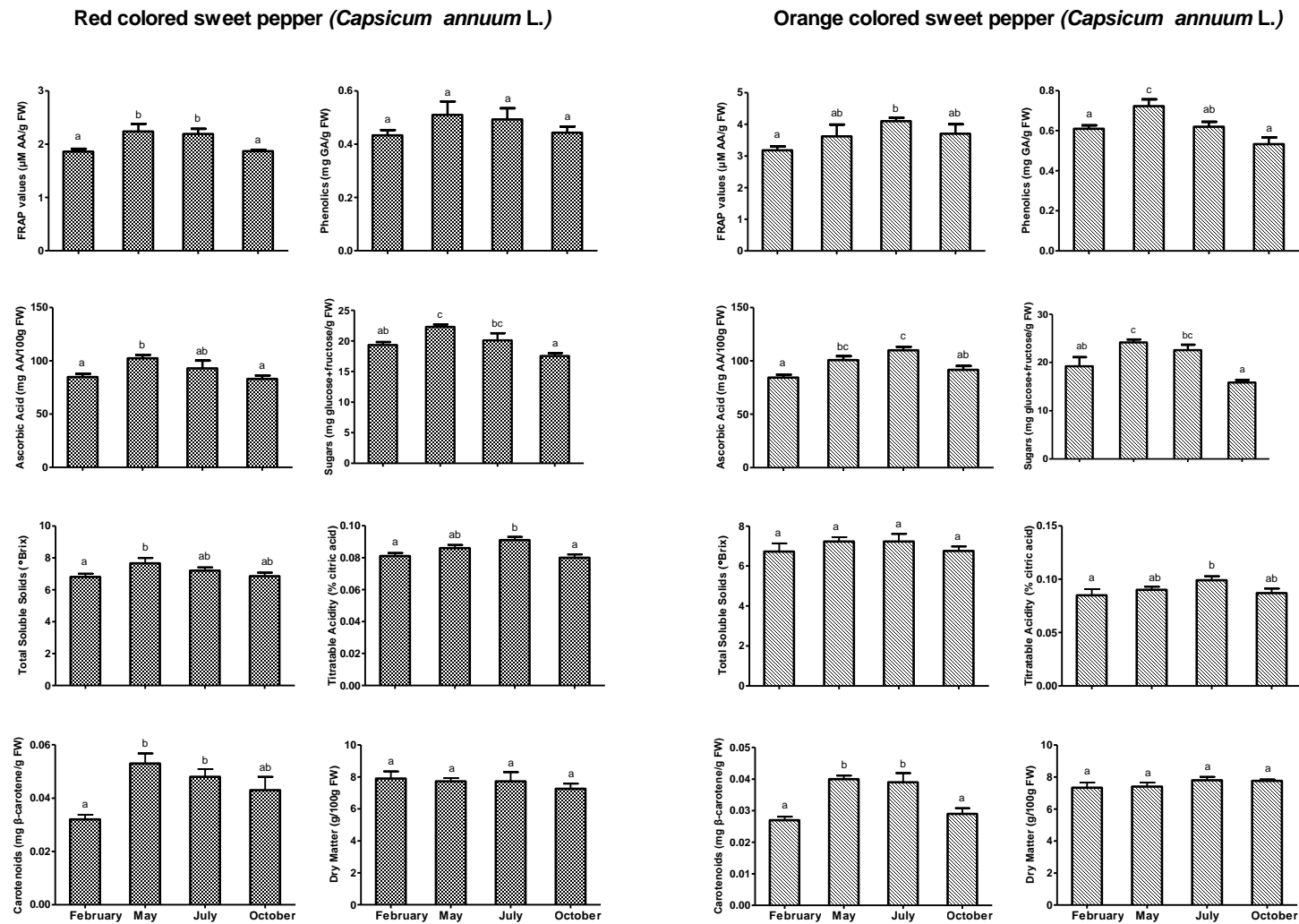


Figure 3. Cont.

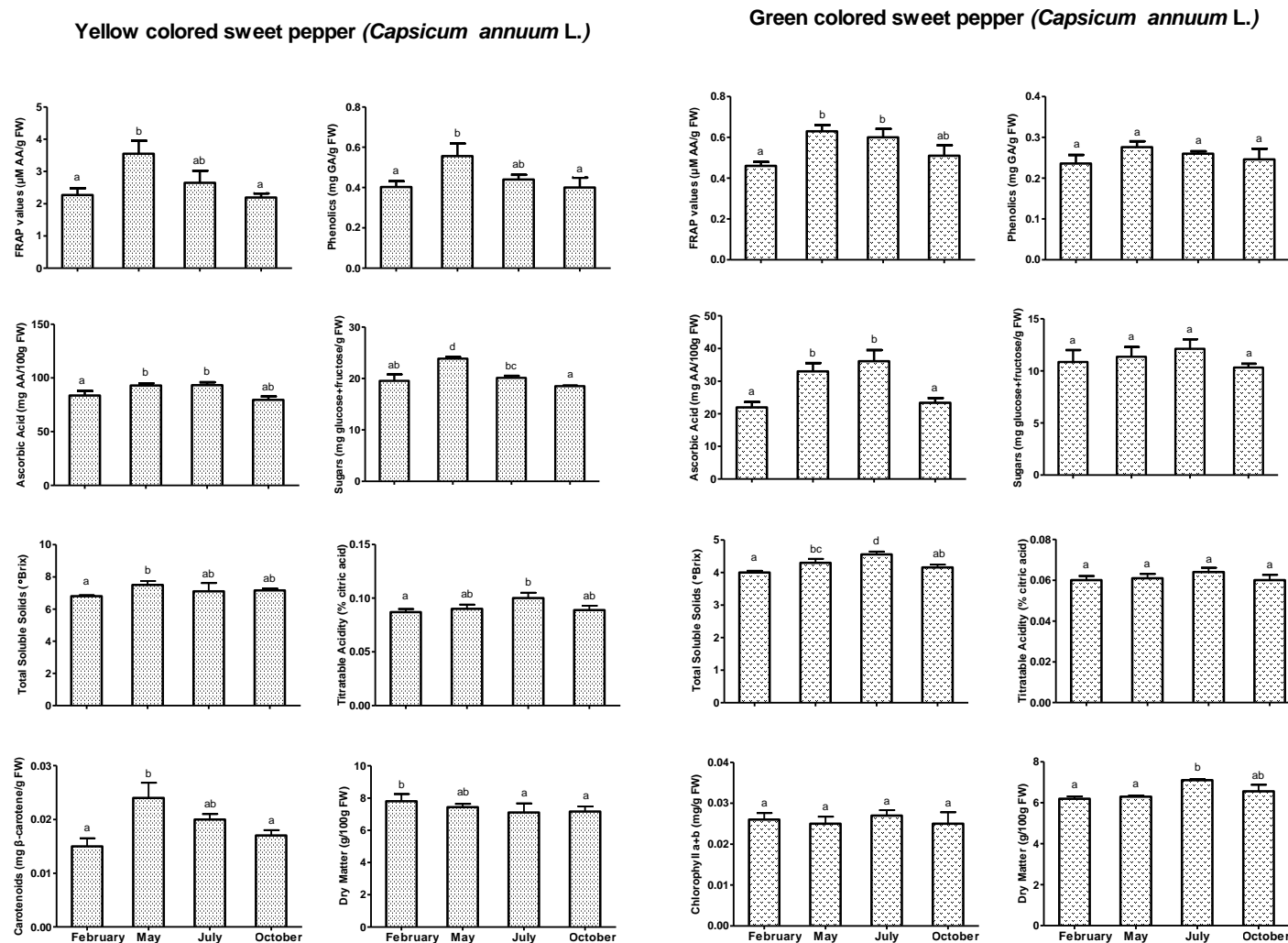


Figure 3. Antioxidant activity (ferric reducing antioxidant power (FRAP) values), phenolics, ascorbic acid, sugars, total soluble solids ($^{\circ}\text{Brix}$), titrateable acidity, carotenoids, total chlorophyll (a + b) and dry matter changes in red, orange, yellow and green sweet pepper cultivars at four harvest months, February, May, July and October 2017. Different lower-case letters above the bars indicate significant differences between mean values at $p < 0.05$ according to Duncan's test. Error bars indicate \pm standard errors of the mean.

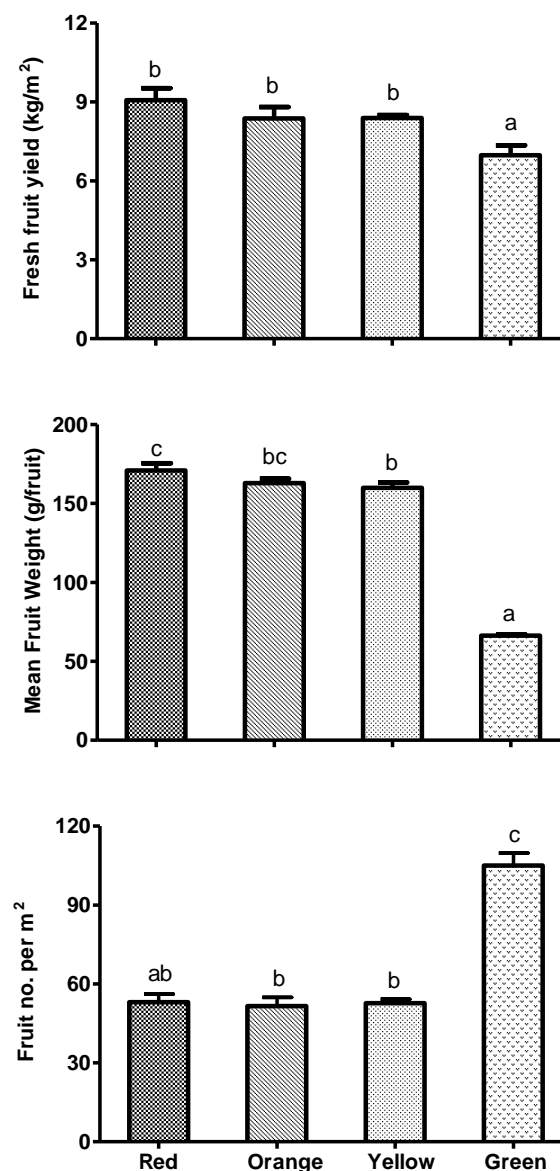


Figure 4. Total marketable fruit yield (kg/m²), average fruit weight (g/fruit) and fruit number per m², in red, orange, yellow and green sweet pepper cultivars from February 2017 until October 2017. Different lower-case letters above the bars indicate significant differences between mean values at $p < 0.05$ according to Duncan's test. Error bars indicate \pm standard errors of the mean.

4. Discussion

Pepper plants were grown in a plastic greenhouse under soilless conditions giving harvests from February until October 2017, to study seasonal variations in fruit antioxidants and antioxidant activity in cultivars of red, orange, yellow and green color. Experimental results on other crops such as spinach and tomatoes demonstrated that antioxidant activity and phytochemicals including phenolics and ascorbic acid were greatly affected by the growing season [14]. In this study, total antioxidant activity and major antioxidant components including, phenolics, ascorbic acid, and carotenoids were higher in pepper fruits during harvesting on May and July in relation to the other two harvesting periods (i.e., February and October) depending on the cultivar (Figure 3). This increase was associated with an increase (avg. 30%) of solar and ultraviolet (UV) radiation and elevated temperature conditions inside the greenhouse from the autumn–winter to spring–summer period (Table 1). Ultraviolet radiation (i.e., Guv values) followed seasonal variations of global solar radiation (i.e., Gh values) as affected by the

yearly length of a day and the solar zenith angle Figure 2; [27]. In this context, literature suggests that light intensity is closely related with the biosynthesis of bioactive (i.e., biologically active) compounds of secondary metabolism in plants such as phenolics, because light increases the activities of key enzymes in the phenolic synthesis such as phenylalanine ammonia lyase (PAL) [14]. It is also known that the synthesis of secondary metabolites in plants is involved in the defense mechanism against several stresses such as UV (280–400 nm) radiation [11]. In accordance, other authors [9] reported phenolics accumulation in different fruits and vegetables in response to UVB (280–315 nm) exposure due to increase expression of phenylpropanoid pathway genes. For example, UV-treated sweet pepper plants contained higher amounts of bioactive compounds such as phenolics so as soluble carbohydrates and photosynthetic pigments at earlier reports [24]. In addition, biosynthesis of phenolic constituents with well-known antioxidant properties in *Capsicum annuum* and other *Capsicum* species including flavonoids, quercetin and luteolin [34], were connected to UVB radiation [11]. These irradiance effects on metabolic functions may also be used to explain phenolics changes with growing season in the current study. In absolute values, even though phenolic and ascorbic acid accumulation is greatly affected by pre- and post-harvest factors [16], the values observed in this study were in the same range with those reported in other experiments for hydroponic sweet peppers grown in a Mediterranean type climate [35].

Moreover, light and temperature in the optimal range stimulate photosynthesis, which leads to the accumulation of reducing sugars and soluble solids in the fruits [36]. Indeed, the increase of ascorbic acid in colored pepper fruits of *C. annuum* L. during May and July was accompanied by an increase in reducing sugars and soluble solids, which supports previous findings for other *Capsicum* species, correlating ascorbic acid biosynthesis with light intensity [8] and reducing sugars (ascorbic acid is synthesized from D-glucose) [37]. Overall, ascorbic acid in fruits of red, orange and yellow colors varied in a range of 80–110 mg/100 g FW, which seems to fall close to that reported previously for greenhouse-grown colored sweet peppers in Spain [38]. Noteworthy, the values observed during spring–summer months were higher than 90 mg/100 g FW and the values during autumn–winter months were lower than 90 mg/100 g FW (Figure 3). Taking into consideration that 90 mg/day is the threshold of ascorbic acid recommended daily allowances for adult men set by the Food and Nutrition Board of the Institute of Medicine in the United States as cited in [37], this study clearly shows that the challenge to eliminate nutritional variations all year round of the selected crops is fundamental. Accordingly, growing season affected carotenoids formations in colored pepper fruits, with higher values observed during spring and summer at elevated light and temperature conditions. Earlier studies have clearly demonstrated that greater exposure to sunlight and higher temperature enhances carotenoid biosynthesis (isoprenoid pathway) in fruits [32]. Thus, the physiological mechanism implicated in the differences between seasons in the current is presumably based on biosynthesis of secondary metabolites from carbon skeletons derived from photosynthetic process [36]. Thus, it is reasonable to conclude that pepper fruit biochemistry was upregulated in response to prevailing environmental conditions (light and temperature) as previously suggested [8]. Considering the total antioxidant activity, the higher activity in spring and summer months was in accordance with the elevated concentrations of phenolics, ascorbic acid and carotenoids in pepper fruits, which confirms the close relationship between these antioxidant components with antioxidant activity [35,39] and their synergistic effect [40]. In general, antioxidant activity reflects the cumulative antioxidant function of a food product [13] and may serve as a tool in epidemiological studies [12]. Particularly, peppers had the second highest total antioxidant capacity among 34 vegetables as reported previously [13]. Summarizing, these results let us suggest that in Mediterranean greenhouses during late autumn and winter light conditions, they need to be more carefully managed (Figure 2) to stimulate brightly colored peppers with higher content of phytochemicals. On the other hand, there is a growing interest among vegetable producers to better control pest and diseases using UV-absorbing films as greenhouse material [24,41], however, UV exclusion may lead to lower concentrations of secondary metabolites in plants and deterioration of product nutritional quality [8]. Therefore, it can be hypothesized that

much stricter selection of the greenhouse covers UV blocking or transmitting properties in conjugation with the cultivated crop and production practices (e.g., crop orientation, harvesting time, planting density), would be beneficial to the growers to reduce pesticide use without a negative effect on phytochemical composition of selected crops in Mediterranean greenhouses. Improvement of product quality in soilless cultivations by manipulating nutrient solution composition has also been stated in several cases [42]. Moreover, the use of artificial light sources (e.g., UV light-emitting diodes) in the greenhouse could not be ruled out, however at the moment is of low usability in horticulture due to operating costs and law restrictions [43]. Furthermore, the data set of this study indicated that although variations in total soluble solids and titratable acidity of pepper fruits may exist at different times of the year, the sensory TSS/acid ratio remained unaffected with time. This may suggest that taste quality of peppers would probably not greatly vary among harvest months in any of the cultivars tested, which is of importance for the market value of the product but may not always coincide with the micronutritional quality of the fruits [44]. Yield results also revealed that there is always a need to validate the results of the earlier studies with the new high yielding cultivars, modern growing systems and prevailing environmental conditions. Indicatively, red cultivar showed greater yield and average fruit weight, followed by orange and yellow, with the lowest values observed in the green one. Total marketable fruit yield and mean fruit weight varied from 8.4 to 9.1 (kg/m²) and 162 to 171 (g/fruit), respectively, for colored fruited peppers. In this content, greenhouse pepper production in Spain yields about 7 kg/m² yearly, whereas colored peppers grown in Florida yielded 6.9 to 11.3 kg/m² in a harvesting period from October to March with an average fruit weight from 161 to 212 g/fruit [45].

5. Conclusions

This study clearly shows the challenge to eliminate fruit antioxidant phytochemical variations in yearly grown greenhouse colored pepper crops. It was clearly shown that the total antioxidant activity and major antioxidant components including phenolics, ascorbic acid, and carotenoids tend to accumulate in higher amounts in sweet pepper fruits at harvesting times with higher solar and ultraviolet radiation and elevated temperature (i.e., spring and summer). Collectively, these results indicate that in Mediterranean greenhouses during late autumn and winter, light conditions can be insufficient to stimulate brightly colored peppers with elevated content of antioxidants, thus the antioxidant activity. This further suggests that a proper selection of greenhouse type and cover material in response to plant–light interception in conjugation with the selected crop and cultivation system may be a prerequisite to optimize environmental conditions for plant growth and elevated antioxidant phytochemicals in yearly grown sweet colored peppers in Mediterranean greenhouses.

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